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Abstract. The variation in semen quality serves to determine the fertility potential of Ongole Crossbred (PO) bulls. Semen quality has a correlation to fertility. Individual motility is one of most important factors determining the success of artificial insemination program. The aims of this research were to mapping the bulls who have high and low fertility potential. This variation is used to determine the composition of molecular marker genes for reproductive traits through the RNA-Sequencing method. 12 PO bulls aged 2–8 years were used in this research. The research parameters were volume, concentration, individual motility, viability, abnormality and sexual behaviour. Sexual behaviour data were analyzed using descriptive methods. Semen quality data were analyzed using an unpaired T-test. The results showed that the bulls code with the highest to lowest motility values were PO-5, PO-2, PO-3, PO-6, PO-4, PO-7, PO-9, PO-8, PO-1, PO-10, PO-11, PO-12, respectively. 4 bulls with the highest motility values and the 4 bulls with the lowest motility values had significant differences ($P<0.05$). Therefore, it can be concluded that the two populations of PO bulls with the highest and lowest motility value are different. It can be continued with the RNA-Sequencing method to find the gene composition of the spermatozoa.

1. Introduction

Ongole Crossbred cattle or better known as Peranakan Ongole (PO) is one of the local cattle breeds that are widely kept in Indonesia. PO cattle are scattered in overall region in Indonesia, especially on the island of Java, and are more concentrated in the province of East Java. Most of them are still kept by small breeders. Performance the PO cattle are able to adapt well to various environment stress and adapt well to tropical climates [1] and poor quality feed [2,3]. Based on this, PO cattle have the potential to be developed. The development of livestock by increasing the population is closely related to the development of males in producing good quality semen.

Bulls productivity can be seen from the bull's ability to produce good libido and high quality semen. This will ensure a successful pregnancy. Artificial Insemination (AI) program can increase bulls productivity [4]. The success of AI was dependent on the quality of bull semen especially individual motility. The variation in semen quality serves to determine the fertility potential of Ongole Crossbred (PO) bulls. Semen quality has a correlation to fertility. Individual motility is one of most important factors determining the success of artificial insemination program [5].

Bull fertility is controlled by genetic and environmental factors. Several studies have been conducted to look at the function of several genes in the male reproductive system. However, there are

no reports on genetic markers for fertility of PO cattle [6]. To know the genes controlling bull fertility, preliminary research is needed, namely regarding the potential semen quality rate. The aims of this research were to mapping the bulls who have high and low fertility potential. This variation is used to determine the composition of molecular marker genes for reproductive traits throught the RNA-Sequencing method.

2. Materials and methods

2.1. Animals

The study was carried out on Cattle Breeding Station – Tuban (UPT PT & HMT Tuban) consisting of 12 PO bulls, aged betwen 2 and 8 years. 28 ejaculations collected from these during three week. All of the bulls were reared under similar management based on standard procedure in UPT PT & HMT-Tuban.

2.2. Data collection and analysis

Semen collected was conducted once a week. Semen quality data was determined through directly observation after semen collection. The research parameters were volume, concentration, individual motility, viability, abnormality and sexual behaviour. From the results of the ranking, the 12 PO males were grouped into two, namely four highest males and four lowest males based on individual motility. The two groups were analyzed by using SPSS 24 with an unpaired T-test. Sexual behaviour data were analyzed using descriptive methods.

3. Results and discussion

The results of individual rankings of PO cattle bulls at UPT PT & HMT Tuban based on individual motility values are presented in table 1.

Table 1. Individual ranking of Ongole Crossbred (PO) bulls from highest to lowest based on individual motility values.

Rank	Bulls code	N Ejaculate	Sperm volume (ml)	Individual motility (%)	Sperm concentration ($x10^7$/ml)	Viability (%)	Abnormality (%)
1	PO-5	3	6.43	80.00	203	75.91	1.15
2	PO-2	3	5.73	78.33	167	77.57	1.75
3	PO-3	3	4.83	78.33	93	83.66	1.42
4	PO-6	3	3.67	78.33	151	77.87	2.21
5	PO-4	3	4.27	76.67	86	78.97	1.98
6	PO-7	2	5.60	76.67	113	77.83	2.38
7	PO-9	3	5.00	63.33	105	73.13	1.65
8	PO-8	2	3.08	55.00	75	71.82	2.58
9	PO-1	3	3.85	50.00	225	83.60	1.33
10	PO-10	1	5.00	50.00	40	78.36	2.99
11	PO-11	1	0.60	40.00	46	80.92	2.29
12	PO-12	1	3.00	40.00	76	82.00	2.00

The results showed that PO bulls at UPT PT & HMT-Tuban had sperm volume values ranging from 0.6 ml to 6.43 ml, individual motility of 40% to 80%, sperm concentrations of $40x10^7$/ml to $225x10^7$/ml, viability of 71.82% to 83.66% and the abnormality of 1.15% to 2.99%. The results of the quality of semen from this study vary widely, some of the quality of semen is in accordance with the [7] study that the criteria for good quality semen are concentrations of more than 500 million/ml,

individual motility more than 65%, abnormal less than 20%. [8] added a volume of semen between 1 to 8 ml. Many factors caused the differences of sperm concentration in each ejaculation, even within the same breed [8]. The sperm concentration depends on sexual development and maturity, male health, testicular size, age, body weight and feed quality [9]. These factors must be considered in the maintenance of elite males to increase the potential for stable semen production [10]. The increasing age will cause the scrotal circumference to become larger, resulting in more semen volume. [11] stated that the higher the scrotal circumference, the more seminiferous tubules, so that semen production increased.

The results showed that out of 12 PO bulls evaluated, four bulls were selected with the highest ranking as the high individual motility group. four bulls with the lowest rank as the low individual motility group. The results of the unpaired T-test showed that these two groups had significant differences ($P<0.05$) (table 2).

The results showed that the high individual motility group was above the minimum value 70% (Indonesian National Standard) for processing into frozen semen. While the low individual motility group was below the minimum value so that it could not be processed into frozen semen. From the results above shows that there are differences so It can be continued with the RNA-Sequencing method to find the gene composition of the spermatozoa. RNA-seq is a technology-based sequencing technique that uses next generation sequencing to reveal the presence and quantity of RNA in a biological sample [12].

Table 2. The average of the individual motility groups were high and low.

Group	N	Mean	Std. Deviation	Std. Error
High individual motility	4	78.75^{b}	0.84	0.42
Low individual motility	4	45.00^{a}	5.77	2.89

^{ab}Different superscripts within columns indicate significant differences ($P<0.05$).

Table 3. The libido of Ongole Crossbred (PO) bulls groups were high and low.

Group	Bulls Code	False mounting	Erection quality	Erection time	Clamping force	Thrust
High	PO-5	1	2+	3+	3+	3+
	PO-2	2	3+	2+	3+	3+
	PO-3	2	3+	3+	3+	3+
	PO-6	2	3+	2+	3+	3+
Low	PO-1	1	3+	3+	3+	3+
	PO-10	2	2+	1+	3+	3+
	PO-11	2	1+	2+	2+	2+
	PO-12	1	3+	2+	3+	3+

The results of observations of libido from 2 individual motility groups can be seen in table 3. The smaller the false mounting value, the better the bull libido assessment. The higher the value of erection quality, erection time, clamping force and thrust, the better the libido. The maximum value is 3+ (kissing, mounting/up, and penis out) [13]. Sexual behavior reflects the ability of bulls to initiate sexual activity as a reflection of the reproductive appearance of livestock [14]. Libido affects semen production and quality. Factors that can cause low libido or sperm quality include handling stress on bulls, age of bull, semen collection technique and environmental [15–17].

4. Conclusions

It can be concluded that the two populations of PO bulls with the highest and lowest motility value are different. It can be continued with the RNA-Sequencing method to find the gene composition of the spermatozoa.

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